Improving Animal Models for Regenerative Medicine

Bethesda, MD

May 23-24, 2012

Meeting Summary

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A. Overview

On May 23 and 24, 2012, the NIH convened a workshop, entitled “Improving Animal Models for Regenerative Medicine.” The goals of the workshop were to: 1) reviewthe current status and benefits of the use of animal models for cell-based regenerative medicine, with the eventual goal of moving these techniques into clinical practice; and 2) for subject matter experts to provide advice to the NIH regarding potential initiatives that will advance the field.

Dr. James Thomson (University of Wisconsin, Madison) began the event with a keynote presentation on primateand human stem cells and nonhuman primates as models for human pluripotent stem cell-based therapies.

The remainder of the 2-day workshop consisted of topical sessions related to regenerative medicine using animal stem cells and models. Each session included individual presentations, followed by a round table discussion and the opportunity for audience participation. Topics of the sessions were:

- Stem cell biology in vertebrate model systems: Cross-species similarities and differences
- New and emerging technologies in stem cell transplantation
- Specific disease modeling and tissue repair
- Challenges of stem cell therapy
- Clinical translation of animal models

Overall, the workshop explored the following major technical areas:

- The biology of animal stem cells – including differences and similarities with human stem cells – which can affect the translation of animal model results into clinical practice.
- The best approaches for isolating, expanding, and reprogramming specific populations of animal stem or progenitor cells for use in understanding or treating specific diseases.
- The application of new technologies: For characterizing animal stem cells and their microenvironment for use in regenerative medicine; improving quality assessment to determine the exact cellular state; and development of non-invasive technologies to monitor engraftment in vivo.
- The use of human stem cells in animals and chimeric animal models to both understand human stem cell biology and accelerate the move toward clinical translation.
- Methods for defining criteria that will assist in choosing the most appropriate animal species and the best animal models for testing new regenerative therapeutics for a variety of disease conditions.

B. Introduction

Regenerative Medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to damage or congenital defects. Importantly, regenerative medicine has the potential to solve the problem of the shortage of organs available for donation. It also holds the promise of repairing or replacing damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves. The recent discovery of the reprogramming of adult cells to a pluripotent state addresses the major problem of regenerative medicine, immune rejection of transplanted tissue. The ability to generate differentiated cells and tissues using patient cells will facilitate individualized medicine and eventually will lead to specialized therapies. The field is moving toward translation to clinical practice and is becoming increasingly dependent on animal models and information regarding potential therapeutic

efficacy of new technologies. Generating the correct type and quantity of the specific cell types required for replacement therapy is a significant challenge, as is the problem associated with getting these cells into the proper environment *in vivo*. Finding solutions to these problems will require extensive *in vivo*testing in experimental animal models.

C. Summary of Presentations

Keynote Speaker: James A. Thomson (University of Wisconsin, WI), "Human pluripotent stem cells: When are non-human primate models needed?"

Dr. Thomson provided an overview of the development of primate embryonic stem (ES) cells. He also presented the overall general process of differentiation of human ES and induced pluripotent stem (iPS) cells into neural, blood, and muscle cells. He pointed outthat there is a significant difference between ESand iPS cells. The reasons forthis are not completely understood.Dr. Thomson explained that CG-DMRs (Differentially Methylated Regions) can reflect both somatic memory and iPS cell-specific methylation. In fact, hundreds of CG-DMRs were present in studies of an iPS cell line (vs. ES) including iPS cell-specific methylation ("iDMR") found in neither ES cells nor progenitor cells. Some CG-DMRs are shared between iPS cell lines.

Nonhuman primate models can be justified in some research areas. For example, these models are helpful in transplantation studies to assess long-term efficacy and safety. They are also helpful in immunological studies to assess the effectiveness of different strategies for preventing immune rejection. Such models may also be helpful in limited *in vivo* developmental studies to demonstrate that *in vitro* human iPS/ES cell results may have *in vivo* relevance. The latter may be useful to determine the impact of cumulative mutations in the transplanted iPS and ES cells and to determine the potential clinical impact of such mutations. Dr. Thomson concluded his presentation by describing the research needs for nonhuman primate iPS cell models.

Session 1 - Stem Cell Biology in Vertebrate Model Systems: Cross-Species Similarities and Differences, Chair Michael Roberts (University of Missouri)

Jose Cibelli (Michigan State University, East Lansing, MI) discussed the search for the best animal models for stem cell research. The mouse model has been thought by some researchers to be the "gold standard" with regard to some studies involving ES cells. While research has been undertaken using porcine, canine, bovine, sheep and goat models for EScell studies each of these models is thought to have specific limitations. More specifically, in the porcine model there are currently no standard culture conditions and the morphology and cell markers are not well defined. In addition, some reports described chimera formation but notgermline transmission. Studies using canine models have shown mixed results, with one canine model showing no teratoma or chimera formation.Work on bovine models has shown particular challenges such as the fact that growth factors required for cow ES cell self-renewal still remain unclear. While the mouse model can show advantages over these models it also still presents limitations in some areas.

Evan Snyder (Sanford-Burnham Institute for Medical Research, La Jolla, CA) focused on stem cell interventions for a range of neurological diseases. Selecting an animal model for a specific neurological condition hinges on a variety of considerations. Some studies, such as those surrounding neurovascular patterning, may not require an animal model. However, both rodent and large animal models (including nonhuman primates) continue to be critical for the study of Parkinson's disease, spinal cord injury, *in utero* interventions, and motor neuron degeneration.

For other conditions rodent models may be sufficient (e.g., lysosomal storage diseases, primary and metastatic brain tumors, stroke and hypoxic-ischemic injury, and Amyotrophic lateral sclerosis). Large animal models offering the closest biology and anatomy relative to humans, can be used to validate safety in clinical trials, and can be optimal for testing the efficacy of molecular, pharmacological, and/or cellular interventions.

Kenneth Chien (Harvard University, Cambridge, MA) discussed the use of stem cell therapy in the regeneration of damaged cardiac tissue. His laboratory has used both murine and rabbit models to explore an approach using chemically modified mRNA (modRNA). The localized, transient, and efficient delivery to the heart of paracrine factors (which control the expansion and differentiation of resident heart progenitors) might represent a viable alternative therapeutic strategy, akin to the known clinical utility of cytokines in selectively augmenting specific blood cell lineages. Results of the studies show that VEGF-A modRNA represents a novel heart progenitor cell fate switch following injury. This may provide a new cell-free therapeutic paradigm to achieve *in vivo* recruitment and subsequent differentiation of endogenous heart progenitors for cardiovascular regeneration.

Ina Dobrinski (University of Calgary, Calgary, Canada) discussed potential therapies using spermatogonial stem cells (SSCs), such as transplantation to restore fertility in cancer patients following chemotherapy as well as the potential of differentiating SSCs into spermatids/sperm *in vitro* to assist infertility patients. Work with these cells in humans is limited by the scarcity of normal human testis tissue for research. However, work in rodent models has elucidated several important pathways. Studies in large animal models (porcine models) have facilitated the investigation of pathways that are conserved in these species.Trans-differentiation studies with porcine SSCs will allow optimization of conditions for subsequent use with human SSCs. Dr. Dobrinski's laboratory has also studied an enzyme called UCH-L1, which seems to be involved in SCC fate decisions and is expressed in the testes of higher mammals.Spermatogonial stem cells can be a good target for germline genetic modification. Also, because these cells have an intermediate position between embryonic and somatic stem cells, they can be a good source of pluripotent cells for regenerative medicine.

Jennifer Gori (University of Washington, Seattle, WA) described the use of the pigtail macaque *(M. nemestrina*) as an excellent model to study long-term engraftment of gene-modified hematopoietic stem cells in an autologous settingdue to the physiological and immunologic similarity of monkeys and humans. More specifically, macaquescan be used as models for using stem cell therapeutics for the treatment of infectious diseases such as hepatitis C and simian AIDS. The testing of new cell therapeutics in such diseasesfirst requires one to scale-up and optimize differentiation before carrying out engraftment studies.Dr. Gori's laboratory developed several iPS cell lines from the pigtail macaque and differentiated them using the embryoid body method to improve hematopoietic specification and expansion of CD34+ cells in order to produce sufficient CD34+ cells for engraftment.Work is underway to test and develop virus-resistant hepatic cells for transplantation as a potential therapy against hepatitis C infection.

Session 2 - New and emerging technologies in stem cell transplantation. Chair: Gerald Schatten (University of Pittsburgh, Pittsburgh, PA)

Jeff W. M. Bulte's (Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD) presentation was devoted to cell tracking to meet the needs of regenerative medicine. To facilitate and implement the translation of novel experimental cell therapies into the clinic, one needs to be able to monitor cellular bio-distribution non-invasively following administration. In humans (as well as in animal models) imaging techniques can be used to track such transplanted cells. Among the different clinically used cellular imaging techniques, the only FDA-

approved method used for imaging infection and inflammation is 111In-oxinescintigraphy. However, cellular magnetic resonance imaging (MRI),which offers superior spatial resolution and excellent soft tissue anatomical detail,is emerging as the technique of choice to monitor real-time, image-guided cell delivery, immediate engraftment, and short-term homing. The primary clinical application of MRI cell tracking may not be monitoring cell migration following injection, but rather verifying the actual accuracy of the cell injection itself at the target site in real-time, using MRI-guided injection procedures.In the future, combining MRI and positron emission tomography (PET) imaging may be beneficial to exploit the relative advantages of each system.

Joseph Wu (Stanford University, Stanford, CA) discussed the use of imaging technology in iPS cell therapy for cardiovascular disease. More than 2,500 patients have received ES cells worldwide through more than 100 clinical trials. However, challenges still remain related to the survival and proliferation of the cells, cell migration, tumorigenicity, integration into the myocardium, and other challenges. Some of these challenges can be addressed by using a variety of imaging techniques – such as labeling stem cells with physical probes – or by using reporter genes. Dr. Wu's laboratory addresses questions using the pUB-hFluc-hGFP transgenic mouse model, including studying cell homing to the myocardium, determining the best time to inject stem cells after myocardial infarction, comparing the transplantation of different adult stem cells, studying gene expression patterns and stem cell engraftment and carrying out disease modeling, drug screening, and cell therapy using iPS cells.

Sheng Ding(Gladstone Institute, San Francisco, CA) described a new technology that allowshigh throughput cell-based phenotypic screenings of arrayed chemical libraries to identify and further characterize small molecules that can control stem cell fate in various systems.Recent discovery efforts have advanced the ability and understanding to control self-renewal, survival, differentiation, and reprogramming of pluripotent stem cells.Studies have shown that some synthetic small molecules can functionally replace transcriptions factors, enhance efficiency, and accelerate speed of reprogramming. Identification of these small molecules can allow one to better understandthe mechanisms underlying reprogramming.

Michele Calos (Stanford University, Stanford, CA)discussed the efforts of her laboratory to develop novel gene and cell therapy strategies to improve the clinical condition of muscular dystrophy patients. The goal is to create iPScells from patient fibroblasts and then correct the dystrophin mutation. The engineered iPScells are differentiated in culture into satellite-like muscle precursor cells, sorted, and transplanted back to the patient.Using the mouse model for Duchenne muscular dystrophy (DMD), iPScells were generated from *mdx*mouse fibroblasts by inserting the wild-type dystrophincDNA using a site-specific recombinase approach. The corrected iPScells are differentiated in cell culture and injected into the muscle of the mouse for engraftment, which is analyzed over time by using luciferase live imaging. Larger animal models will be employed as the study progresses (e.g. for DMD, a dog model exists with a similar pathology to the human disease). A porcine model is also being considered.

Eric Ahrens (Carnegie Mellon University, Pittsburgh, PA)discussed new Magnetic Resonance Imaging (MRI)applications, which are experiencing a rapid expansion in theability to visualize specific cell populations and molecular events *in vivo*. He described novel perfluorocarbon (PFC) emulsions to label cells *ex vivo*. When these labeled cells are introduced into the subject, their migration can be monitored using fluorine-19 (19F) MRI.Resulting images are extremely selective for the labeled cells, with no background signal from the host's tissues. Moreover, the absolute number of labeled cells in regions of interest can be estimated directly from the*in vivo* images.These unique tools are being used in preclinical studies to elucidate the etiology and dynamics of inflammatory events in cancer and autoimmune diseases. Additionally, the PFC emulsion reagents have bio-sensing properties that report on the absolute level of intracellular

oxygen and can potentially monitor cell activation, differentiation, or apoptosis *in vivo*.Efforts are underway to characterize new generations of nucleic-acid based MRI reporters. For example, MRI reporters can be used for labeling stem cells for long-term tracking, or for imaging transgene expression in genetically-manipulated animals.

Session 3 - Specific disease modeling and tissue repair. Chair: Jose Cibelli (Michigan State University, East Lansing, MI)

Maike Sander (University of California, San Diego, CA) focused on the major challenge for developing regenerative therapies for diabetes, the limited capacity of the adult human pancreas to generate new beta-cells.Research bottlenecks include the inability to culture human cadaver beta-cells in vitro, the lack of suitable assay systems to study beta-cell replication *in vitro* or *in vivo*, as well as the inability to measure beta-cell mass in live animals., Efforts in the field have focused on replacing lost beta-cells in diabetes by deriving functional beta-cells from human pluripotent stem (hPS) cells due to the limitations in the ability to expand human beta-cells. Although it isstill not possible to generate fully functional beta-cells from hPS cells*in vitro*, much progress has been made. The challenge lies in studying these cells after transplantation and in designing meaningful studies of how hPS-derived beta-cells interact with the human immune system in type 1 diabetes. Animal models that appropriately mimic the disease and allow for studies of cell-immune system interactions are limited and additional models are still needed for testing future cell therapies for type 1 diabetes.

ShoukhratMitalipov (Oregon Health and Science University, Oregon National Primate Research Center, Beaverton, OR) described ground-breaking studies demonstrating that rhesus monkey EScells failed to incorporate into host embryos and develop into chimeras.However, freshly isolated cells of the inner cell mass (ICM) transplanted into blastocysts formed separate viable fetuses while sharing the placental compartment of the host embryo. Classical embryo chimeras were produced by aggregation of totipotent cells of the 4-cell monkey embryos. The Mitalipov laboratory is also investigating novel gene therapy approaches for the treatment of human diseases. Mutations in mitochondrial DNA (mtDNA) contribute to a diverse range of incurable human diseases and disorders,including neurodegenerative diseases, myopathies, diabetes, cancer and infertility.These researchers have recently demonstrated that the mitochondrial genome can be efficiently replaced in mature nonhuman primate oocytes by spindle-chromosomal complex transfer from one egg to an enucleated, mitochondrial-replete egg. The reconstructed oocytes with the mitochondrial replacement were capable of supporting normal fertilization and embryo development and produced healthy offspring.The overall goal of this project is to replicate monkey studies with human oocytes donated by patients carrying mtDNA mutations after informed consent.

Marina Emborg (University of Wisconsin, Wisconsin National Primate Research Center, Madison, WI)discussed modeling of Parkinson's disease (PD). Treatment therapies for PD will include clinical translation of cell-based strategies for brain repair. This has been modeled by using neurotoxin-induced nonhuman primate models of PD to perform preclinical evaluation of invasive and first-in-class therapies. Different cell types have been proposed as replacements of dopaminergic nigral cells lost in the disease and also as potentialsources of therapeutic agents. Recent breakthroughs incell biology are helping to develop novel cell lines that could be used for regenerative medicine. Their future clinical application depends on identifying and solving problems encountered in previous trials. There are several animal models for PD including yeast (genetic), *drosophila* (genetic), *C. elegans* (genetic, toxic), fish (toxic), cats (toxic), pigs (toxic), and nonhuman primates. To test potential effects of human cell transplantation, human neural precursor cells-Glial cell line-derived neurotrophicfactor (GDNF) has been transplanted into MPTP-treated nonhuman primates.Human neural precursor cells-GDNF induced astrocyteand microglia responses, whereas autologous primate iPS cells have been found to

survive for up to six months without immunoreactivity.The speaker concluded thatthe best model that should be used in a particular study should dependuponthe specific research question under investigation.

Kang Zhang (University of California, San Diego, CA; UCSD) discussed the leading causes of irreversible visual impairment, which includes age-related macular degeneration (AMD), retinal vascular diseases, and glaucoma.Studies show that allelic variants of genes encoding members of the alternative complement pathway – including CFH and C3 – strongly influence an individual's risk of developing AMD. Work at UCSD and other laboratories has demonstrated that the HTRA1 gene at chromosome 10q26 also strongly impacts AMD risk.The group at USCD has derived iPS cellsfrom patients from high risk genotypes for AMD and glaucoma and has differentiated them into retinal neurons in order to model disease phenotypes and study mechanisms *in vitro*and in animal models. ES cell derived human neural stem cells have been shown to preserve photoreceptors and visual functionwhen introduced into experimental animals. Also, visual preservation in treated eyes has shown near normal visual function.Because mice do not have maculas, there is an urgent need to create mini pig and nonhuman primate models for macular degeneration.

Fumihiko Ishikawa (RIKEN Research Center for Allergy and Immunology, Yokohama, Japan) described developmentof humanized mouse models by intravenously injecting purified human hematopoietic stem cells (HSCs) into immune-compromised newborn mice (NOD/SCID/IL2rgKO). This xenogeneic transplantation system allows for the long-term engraftment of human HSCs. In recipient organs, studies showed the physiological development of multiple lymphoid and myeloid progeny that exhibited immune function *in vivo*. This model is expected to serve as a useful research tool for direct investigation of human immune function.In addition to understanding human hematopoiesis and immunity, creation of humanized mice may also facilitate translation of research findings into therapeutic and pharmaceutical development. As a possibility for such translation, the RIKEN Research Center for Allergy and Immunology has also developed *in vivo* models of human leukemia, Epstein Barr virus infection, and primary immunodeficiency.

Session 4 - The potential challenges of stem cell therapy. Chair: Joseph Wu (Stanford University, Stanford, CA)

Michael Roberts (University of Missouri, Columbia, MO) described the studies in his laboratory devoted to iPScells from swine. The porcine model has been used in various areas of biomedical research, including studies of xenotransplantation, myocardial infarction, cerebral ischemia, intestinal metabolism, asthma, osteoporosis, and other conditions. iPS cell lines have been readily generated from pigs by several groups of researchers. The porcine iPScells generated in this manner resemble human ES and iPS cells rather than their murine equivalents.More recently it has become possible to derive cells with a so-called naïve phenotype (similar to mouse ES cells). In theory, porcine iPScells could be "personalized" to specific pigs, and such animals could later be used to test transplantation therapies for safety and efficacy prior to applying such procedures to humans. There is also potential value in using iPScells to developing transgenic pigs. Genetic changes, particularly ones that are complex, may be readily selectable. Also, nuclei from pluripotent cell lines may be easier to reprogram than somatic cells within oocyte cytoplasm. Nuclei from pluripotent cells may carry less of an epigenetic memory than nuclei from somatic cells. In conclusion, the speaker stated that well developed technologies for introducing genetic changes into the pig are currently available. Facilities and resources for biomedical research using pigs, however, are still not widespread.

Mahendra Rao (National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH) focused on regulation of cell based therapies by the US Food and Drug Administration.Human

cell/tissue products that are cultured or manipulated and are not intended for homologous or allogeneic use, require regulation through Section 351 of the Public Health and Safety Act. Cell therapies, whether regulated or not,usually require animal models for testing. Animal testing can be performed to study proof of concept,biodistribution,safety and immune issues. Studies involving ES or iPScells can at times require that specific issues be addressed with regard to animal models. For example, cell products can provoke an immune response for a variety of reasons (e.g. use of fetal cells, xeno components used in manufacture, ancillary components such as scaffolds, or cross reactive antigens in tissue). This has resulted in alternative research strategies including *in vitro* testing as well as the development of humanized models, locating immunoprivileged sites, and using immunocompromised animal models.

Yang Xu (University of California, San Diego, CA) described the efforts to overcome allogeneic immune rejection of human iPS (hiPS)cells by transplant recipients. Dr. Xu's laboratory used an existing humanized mouse (hu-mouse) model to study the mechanism of human immune responses to the derivatives of human pluripotent stem cells and to test the hypothesis that the immunogenicity of these cellsisa functional readout of the epigenetic and genetic abnormalities found in iPS cells.The model was modified as follows: human fetal liver/thymus was transplanted intoNOD scid IL2 receptor gamma chain knockout(NSG) mice; fibroblasts were derived from the fetal liver of the same donor; these fibroblasts were then reprogrammed into hiPS cells; and the hiPScells were transplanted back into the hu-mice. This created an autologous system. Using this modified model, the group at the University of California, San Diego carried out various studies to test the immunogenicity of hiPS cells.Studies on this model showed that cells derived from autologous hiPS cellsare much less immunogenic than those from allogeneic human ES (hES) cells. Epigenetic aberrations were found to directly contribute to the immunogenicity of hiPS cellderivatives.

Tobias Cantz (Max-Planck Institute, Hannover, Germany) discussed the challenges and promises of the use of iPS cells in hepatic therapy. The supply of adult hepatocytes from non-transplantable donor organs is still limited and an allogeneic cell-based transplant involves risk of graft loss due to immunological rejection. However, autologous cell sources for hepatic cell replacement and self-renewing stem cells expanded *in vitro* hold promise in thisarea. While the generation of iPS cellsprovides a well-suited cell source for this approach, efficient differentiation protocols are required to achieve hepatic cells with advanced hepatic maturity. Furthermore, the risk of *de novo*epigenetic aberrations should be minimized during the process of stem cell generation, expansion, and differentiation.Dr. Cantz' laboratory investigated the use of lentiviral vectors to correct mutations iniPS cells.In this model, adult hepatocytes showed a higher liver repopulation capacity when compared to fetal hepatoblasts and stem cell derived hepatic progenitors. This study showed that there is an urgent need to improve stem cell differentiation strategies.The consideration of epigenetic aberrations and contamination by tumorigenic cells should be addressed in future pre-clinical studies including large animal models.

Andras Nagy (The Samuel Lunenfeld Research Institute, Toronto, Canada) described studies from his laboratory aimed at designingnovel DNA vectors to aid the interrogation of the role of mouse genes and genomic components."DNA-smart" enzymes, such as recombinases, integrases, transposons, and DNA binders can assist in screening a genome for critical units. Possible applications include: 1) NorCOMM targeted alleles used to generate replaceable mutations in more than 600 genes during a high throughput knockout project; 2) Several docking site designs utilizing the PhiC31 integrase for site-specific transgene integration; and 3) Applications based on the efficiency of DNA element transposition using the piggyBactransposon system.Work is underway to develop a new set of biologics to treat Age-related Macular Degeneration (AMD). These biologics would aid in "mopping up" excess VEGF at the site of expression and would act locally. The approach involves introducing doxycycline

transgenes into stem cells to create a therapeutic cell type that acts as a VEGF trap. These cells would then be transplanted into the eye and eliminate the need for chronic eye injections. Both iPS cellsand ES cellscan be used for this approach. Each of them offers specific differences in the following areas: epigenetics, genome integrity, pluripotency, differentiation, disease modeling, safety, immunogenicity, and economics.

Session 5- Clinical translation of animal models. Chair: Mahendra Rao (NIH)

Roxanne Reger (Texas A&M Health Sciences Center, Temple, TX) described the use of mesenchymal stem/progenitor cells (MSCs) in animal models for regenerative medicine. These cells can differentiate into fat, cartilage, bone, neural and other phenotypes and have demonstrated beneficial effects in a broad range of diseases and conditions in animal models including diabetes, stroke, myocardial infarction, and multiple sclerosis.The interest in MSCs is reflected by more than 150 clinical trials, some of which have progressed into Phase III trials. One disadvantage is that MSCs have been found to be sensitive to their micro-environments and can undergo changes when expanded in culture based on density, medium, and other undefined variables. Because there are no convincing tests for efficacy – or consensus about reliable markers for cells – large differences can exist in the properties of MSCs prepared by different laboratories.To address this variability, Texas A&Mis funded by the Office of Research Infrastructure Programs, NIH, to provide well-characterized human and rodents adult stem cells. To date, the laboratory has made 412 shipments of MSCs to more than 280 investigators. Recommendations were given to improve current services and provide therapeutic grade MSCs to biomedical investigators.

Trista North (Harvard University, Cambridge, MA)presented new approaches for developing chemical screen derived therapeutics for regenerative medicine in a fish model system. As an example, prostaglandin E2 (PGE2) – the first compound derived from *in vivo* chemical genetic screening in the zebrafish model – has successfully concluded Phase I trials.A Phase I trial at the Dana-Farber Cancer Institute examined transplantation of umbilical cord blood after short-term *ex vivo* exposure to dmPGE2 (a stabilized version of PGE2). Findings demonstrated that cord blood treated with dmPGE2 enhanced engraftment after transplantation and reduced time to neutrophil recovery,a clinical endpoint indicative of patient outcome. Multi-center Phase II trials have been initiated. In addition to transplantation therapy, zebrafish chemical screens have also led to cancer (metastatic melanoma) and toxicology therapeutics (acetaminophen liver toxicity) that are entering clinical testing.

Henry Kaplan (University of Louisville, Louisville, KY) described development of the inbred miniature pig model of Retinitis Pigmentosa (RP). Using somatic cell nuclear transfer, scientists at the University of Louisville have developed an animal model using the miniature pig that expresses the human RHO P23H transgene.Six transgenic miniature pigs were developed. Offspring from one of the most severely affected founders inherited the transgene in Mendelian fashion as an autosomal dominant mutation and demonstrated rod photoreceptor dysfunction at birth with progressive rod and cone degeneration over time. Histology and other results suggest that the miniature pig model mimics many of the features of RP and may serve as a novel tool for study of pathogenesis and therapeutic intervention in the most common form of hereditary retinal degeneration.The pig model allows for microarray analysis, gene therapy (gene modification), and selected pharmacologic targeting of molecular pathways to enhance stem cell transplantation and photoreceptor survival.Several obstacles were highlighted in the presentation which are limiting the development of effective therapeutic approaches against RP, such as insufficient focus on molecular pathways leading to cell death, absence of visual behavioral tests for large animals, and limited knowledge of the best window of opportunity for the intervention.

Martin Marsala (University of California, San Diego, CA)discussed a miniature pig model for the preclinical development of cell replacement therapies. Porcine models for preclinical safety and efficacy in brain and spinal cord studies offer several advantages over other models, including cost and similarities in the dimensions of the spinal cord/brain compared to humans. Successful use of porcine models requires the following: effective use of immunosuppression protocols; availability of porcine derived neural cell lines; development of appropriate delivery systems; and availability of neurodegenerative models and inbred strains.
Using human fetal spinal cord-derived neural precursors (or human embryonic stem cell-derived neural precursors) scientists have used the above model to characterize the optimal cell dosing regimen that is safe and well tolerated over an extended period of time following spinal or intra-striatal cell grafting. The model has also been used to develop an immunosuppression protocol thatpermits long-term xenograft survival.The data from these preclinical studies have been used to submit anInvestigational New Drug(IND) application for treatment ofamyotrophic lateral sclerosis(ALS) by spinal grafting of human fetal tissue-derived neural precursors. This animal model may also be used for a variety of neurodegenerative disorders, including, brain and spinal cord ischemic injury, or spinal trauma.

Sowmya Viswanathan (University of Toronto, Toronto, Canada) described the Cell Therapy Program (CTP) at the University of Toronto Health Network. It includes a translational laboratorythatfocuses on proof or principle, toxicology, and safety studies. Cell manufacturing facilities are also in place and used to manufacture clinical grade cells. Eight trials have been (or are currentlybeing) held in a variety of areas, including autologous stem cell transplantation, regenerative medicine andoncology.Three studies were presented as examples in a preCTA meeting with Health Canada (similar to a pre IND meeting in the U.S.). Efforts are also underway for a preclinical study on mice thataims to correct Fabry disease through gene therapy. The speaker also stressed that there is no best single animal model for immune response, disease modulation, dose prediction, etc. suitable for all cases of cell based therapy. All models have pros and cons. A case-by-case assessment needs to be undertaken to determine the best model for the research question being asked.

D. Recommendations

1. Animal stem cell biology needs to be a focus of future research to understand stem cell maintenance and reprograming requirements for cells from a variety of animal species. Animal models can be used to study human disease conditions as well as to search for new therapeutic approaches for regenerative medicine. To progress in this direction:
    - Stable, well characterized pluripotent stem cell lines from large animal species, such as rabbits, pigs, sheep and monkeys, should be created and made available to the biomedical community. Biomarkers and standard protocols should be created to maintain and characterize the states of these cells.
    - Efficient protocols should be developed for scale-up of cell production and efficient cell reprogramming.
    - Species-specific reagents, such as antibodies and microarrays, should be developed and be madecommercially available. There is a need for development of the proteomic and genomic tools that will assist the use of stem cells from large animal species, like rabbits, pigs, sheep and monkeys.

- Approaches for endogenous stem cell *in vivo* reprogramming should be developed and tested as well as transdifferentiation methods for somatic cells. The approaches should include temporary delivery or induction of transcription factors as well as small molecule-mediated reprogramming.

2. The behavior and the fate of different animal and human stem and progenitor cells as well as their derivatives upon introduction to the whole animals should be investigated. Efforts should be made to increase cell survival after cell-grafting experiments. These studies should provide information on the criteria for selecting the best type of stem cells for a particular application. These studies are critically dependent on the further development of methods and advanced technologies to follow and identify the introduced populations of cells. Further characterization and improvement of humanized animal models, including both rodents and large animals, such as pigs, should have a significant impact on the field.
3. Germ line stem cell research using animal models shouldbe enhanced due to the potential to introduce genetic changes to be transmitted to the next generation as well as opportunities to study functional end points that are not possible to investigate in humans. Applications for regenerative medicine and human infertility should be considered.
4. Further investigation of problems already identified as affecting safety and efficacy of stem cell mediated therapies should be of high priority. These areas include genetic instability and high mutation rate after *in vitro* manipulations, epigenetic memory of differentiated iPS cells and immune responses induced after stem cell transplantation. These issues requirefurther evaluation and the search for solutions in relevant animal models with further confirmation using human stem cells.
5. Further progress should be made in development of effective gene-therapy approaches using *ex-vivo* engineered stem cells and their derivatives as well as the introduction of genes that correct genetic defects into the stem cell populations *in vivo*. These approaches should be compared and those that provide sustained genetic repair moved to pre-clinical trials and eventually to the clinic.
6. Animal models for cell-mediated therapies for a variety of human disease conditions required further improvements. Special attention should be paid to informative models for the following animal species:
    - For minipigs: i) Strain-specific T cell mediated rejection should be further characterized; ii) Immunosuppression protocols for xenotransplantation experiments should be improved; iii) Tests for monitoring humoral immunity should be developed; iv) Standardized iPS cells should be developed and distributed; v) Availability of the animals themselves should be increased.
    - For Zebrafish: Animals, protocols and reagents should be made widely available both to the zebrafish community and to other laboratories that may want to use this animal model.
    - For nonhuman primates:i) Transplantation studies should be designed to test issues regarding long-term engraftment and safety; ii) Strategies for preventing immune rejection should be optimized; iii) *In vivo* studies using ES or iPScells should be performed in optimized test systems to examine correlation with results obtained *in vitro.*

7. Databases of reproducible experimental conditions and results obtained from testing of animal and human stem cells in different animal species should be created and shared among laboratories and investigators.
8. There is a need to further developguidance and regulatory requirements for animal studies to assure safety and efficacy of stem cell-based product applications for human therapeutics. Recommendations should provide assistance in the selection of the relevant preclinical animal models, the design of the animal study, expected informative end points, criteria for functional success and other practical considerations.

E.Conclusion

The workshop gathered a diverse group of biomedical experts to evaluate the status of the use of animal and human stem cells in animal models for regenerative medicine. Meeting participants came to the conclusion that further development of animal models for regenerative medicine will aid the development of new treatments for many diseases, which cannot currently be addressed by drug therapy. The use of stem cells presents a variety of challenges, which require innovative research and laboratory validation in a wide range of laboratory animal species. Only by conducting such research can the full potential of regenerative medicine for investigating diseases and treating patients be explored.

Appendix A. Symposium Agenda

DCM/DPCPSI/OD-NIH Symposium,
May 23-24, 2012
Lister Hill Auditorium, NIH, Bethesda, MD

Purpose of the meeting: The **purpose** of the workshop is to convene a colloquium on the current status of and requirements for the use of animal models for cell-based regenerative medicine, with the eventual goal of moving these techniques into clinical practice. Meeting participants will provide insight to the Division of Comparative Medicine and other NIH units for the development of potential initiatives in this rapidly evolving area of research and development. In addition, this meeting will encourage the biomedical community to use animal stem cells for creation of regenerative medicine models.

Organizing Committee: Oleg Mirochnitchenko (OD/NIH), Jack Harding (OD/NIH), Mahendra Rao (NIAMS/NIH), Marina Emborg (University of Wisconsin, WI), Jose Cibelli (Michigan State University, MI), Michael Roberts (University of Missouri, MO), Darwin Prockop (Texas A&M Health Sciences Center, TX), Gerald Schatten (University of Pittsburgh, PA)

The following major directions will be explored:

1. Biology of animal stem cells, differences and similarities with human stem cells which will affect the translation of the animal model results to clinical practice;
2. The best approaches for isolating, expanding and reprograming the specific populations of animal stem or progenitor cells for use in understanding or treating specific diseases;
3. Application of new technologies for characterizing animal stem cells and their microenvironment for use in regenerative medicine. Improving quality assessment to determine the exact cellular state;
4. The use of chimeric animal models to understand human stem cell biology and to move toward clinical translation;
5. Defining the best animal models for testing new regenerative therapeutics, including specifically for nonhuman primates (NHPs): defining appropriate models and technological improvements.

Day 1 – Wednesday, May 23, 2012

8:30 – 9:00	**Introduction and welcome** Symposium Introduction: Oleg Mirochnitchenko/Jack Harding (OD/NIH) Welcome: James M. Anderson, DPCPSI Director
9:00 – 9:45	**Keynote Presentation** "Human Pluripotent Stem Cells: When are non-human primate models needed?" **James A. Thomson** (University of Wisconsin, Madison, WI)

Session 1: Stem cell biology in vertebrate model systems: cross species similarities and differences. Chair: Michael Roberts (University of Missouri, Columbia, MO)

9:45 – 10:05	"Current status of ESC in non-traditional Animal Models: Are there any non-primate, non-rodent ESCs that meet the gold standard set by the mouse model?", Jose Cibelli (Michigan State University, East Lansing, MI)
10:05 – 10:25	"Informative Animal Models for Assessing the Efficacy & Safety of Stem Cells in Neurological Conditions", Evan Snyder (Sanford-Burnham Institute for Medical Research, La Jolla, CA)
10:25 – 10:45	**BREAK**
10:45 – 11:05	"Driving heart progenitor cell fate and regeneration in vivo via chemically modified mRNA", Kenneth R. Chien (Harvard University, Cambridge, MA)
11:05 – 11:25	"A Large Animal Model for Germ Line Stem Cell Research", Ina Dobrinski (University of Calgary, Calgary, Canada)
11:25 – 11:45	"Robust Differentiation and Viral Infection of Pigtail Macaque Induced Pluripotent Stem Cell-derived Hepatic and Hematopoietic Cells. Toward Modeling Human Infectious Disease and Stem Cell Therapies *In Vivo*", Jennifer Gori/Hans-Peter Kiem (University of Washington, Seattle, WA)
11:45 – 12:15	**Round Table Discussion**
12:15 – 13:15	**LUNCH**

Session 2: New and emerging technologies in stem cell transplantation. Chair: Gerald Schatten (University of Pittsburgh, Pittsburgh,PA)

13:15 – 13:35	"Cell Tracking and Regenerative Medicine: Clinical Needs and Technological Solutions", Jeff W. M. Bulte (Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD)
13:35 – 13:55	"Imaging of Pluripotent Stem Cell Therapy", Joseph C. Wu (Stanford University, Stanford, CA)

13:55 – 14:15 "A chemical Approach to Controlling Cell Fate", Sheng Ding (Gladstone Institute, San Francisco, CA)

14:15 – 14:35 "An Engineered iPSC Transplantation Strategy for Muscular Dystrophy", Michele Calos (Stanford University, Stanford, CA)

14:35 – 14:55 "Emerging MRI methods for in vivo cell tracking",Eric T. Ahrens (Carnegie Mellon University, Pittsburgh, PA)

14:55 – 15:25 **Round Table Discussion**

15:25 – 15:45 **BREAK**

Session 3: Specific disease modeling and tissue repair. Chair: Jose Cibelli (Michigan State University, East Lansing, MI)

15:45 – 16:05 "Cell Regeneration and Replacement in Diabetes: Advances and Hurdles", Maike Sander (University of California, San Diego, CA)

16:05 – 16:25 "Rhesus Macaque Model for Stem Cell and Gene Therapies" ShoukhratMitalipov (Oregon Health and Science University, Beaverton, OR)

16:25 – 16:45 "Preclinical Evaluation of Cell-based Therapies for Parkinson's Disease", Marina Emborg (University of Wisconsin, Madison, WI)

16:45 – 17:05 "Genetics and Stem Cell Based Therapies for Blindness", Kang Zhang (University of California, San Diego, CA)

17:05 – 17:25 "Creation of Humanized Mouse Model for Human Immunity & Diseases", Fumihiko Ishikawa (RIKEN Research Center for Allergy and Immunology , Yokohama, Japan)

17:25 – 17:55 **Round Table Discussion**

Day 2 – Thursday, May 24, 2012

Session 4: The potential challenges of stem cell therapy. Chair: Joseph Wu (Stanford University, Stanford, CA)

8:30 – 8:50	"Induced Pluripotent Stem Cells from Swine (*Susscrofa*): Why They May Prove To BeImportant?",Michael Roberts (University of Missouri, Columbia, MO)
8:50 – 9:10	"Issues with Manufacturing Differentiated Cells from Pluripotent Stem Cells for Preclinical Studies", Mahendra Rao (NIH)
9:10 – 9:30	"Humanized mouse Model to Study the Immunogenicity of Human Pluripotent Stem Cells", Yang Xu (University of California, San Diego, CA)
9:30 – 9:50	"Pluripotent Stem Cells in Hepatic Cell Therapies: Challenges and Promises", Tobias Cantz (Max-Planck Institute, Hannover, Germany)
9:50 – 10:10	"DNA-smart" Enzymes for Precise Tailoring of the Mouse Genome", Andras Nagy (The Samuel Lunenfeld Research Institute, Toronto, Canada)
10:10 – 10:40	**Round Table Discussion**
10:40 – 11:40	**LUNCH**

Session 5: Clinical translation of animal models. Chair: Mahendra Rao (NIH)

11:40 – 12:00	"Standardized Preparations of Mesenchymal Stem/Progenitor Cells (MSCs) for Testing in Animal Models for Regenerative Medicine", Darwin Prockop (Texas A&M Health Sciences Center, Temple, TX)
12:00 – 12:20	"Zebrafish Chemical Screen-Derived Therapeutics Enter Regenerative Medicine Clinical Trials", Trista North (Harvard University, Cambridge, MA)
12:20 – 12:40	"Generation of an Inbred Miniature Pig Model of Retinitis Pigmentosa", Henry Kaplan (University of Louisville, Louisville, KY)
12:40 – 13:00	"Brain and Spinal Cell Grafting in Immunosuppressed Miniature Pigs: a Preclinical Model to Evaluate Safety and Toxicity of Human Neural Precursors Cell Lines", Martin Marsala (University of California, San Diego, CA)
13:00 – 13:20 "	The Gap Between Translational and Clinical Research for Cell-Based Therapeutics", Sowmya Viswanathan (University of Toronto, Toronto, Canada)
13:20 – 13:50	**Round Table Discussion**
13:50 – 14:20	**Closing Remarks/Recommendations: Gerald Schatten (University of Pittsburgh, Pittsburgh, PA)**

Appendix B. NIH Scientific Organizing Committee and Participant List

Revised to reflect final attendance.

Scientific Organizing Committee

Jose Cibelli
Michigan State University

Marina Emborg
University of Wisconsin

John D. Harding
Office of Research Infrastructure Programs
DPCPSI, NIH

Oleg Mirochnitchenko
Office of Research Infrastructure Programs
DPCPSI, NIH

Darwin Prockop
Texas A&M Health Science Center

Mahendra Rao
National Incident Management System
NIH

Michael Roberts
University of Missouri

Gerald Schatten
University of Pittsburgh

NIH Participants

Kristin Abraham	Diabetes, Endocrinology & Metabolism, NIH
KapilBharti	National Institutes of Health
Olivier Blondel	National Institute of Diabetes & Digestive & Kidney Diseases, NIH
Henry Chang	National Heart, Lung and Blood Institute, NIH
Amjad Chaudhry	National Institute of Mental Health, NIH
Laura Cole	National Institute on Deafness and Other Communication Disorders, NIH

NIH Participants	
Liz Conner	National Cancer Institute, NIH
Mrinal Dewanjee	National Eye Institute, NIH
Lijin Dong	National Eye Institute, NIH
Yubin Du	National Heart, Lung, and Blood Institute, NIH
Kevin Francis	National Institute of Child Health and Human Development, NIH
Joe Frank	Radiology and Imaging Sciences, NIH
Carol Haft	National Institute of Diabetes & Digestive & Kidney Diseases, NIH
Yi-Xing Han	National Cancer Institute, NIH
Jack Harding	Division of Comparative Medicine, NIH
Buster Hawkins	National Heart, Lung, and Blood Institute, NIH
Susan Haynes	National Institute of General Medical Sciences, NIH
So Gun Hong	National Heart, Lung and Blood Institute, NIH
Tanya Hoodbhoy	OD/DPCPSI/Office of Strategic Coordination, NIH
Ann Jenkins	National Institutes of Health
Mahin Khatami	National Cancer Institute, NIH
Saejeong Kim	Clinical Center, NIH
Okjae Koo	National Institute of Allergy and Infectious Diseases, NIH
Kristy Kraemer	National Institute of Allergy and Infectious Diseases, NIH
MielojKrephisla	National Institute of Dental and Craniofacial Research, NIH
Hongzhen Li	National Heart, Lung, and Blood Institute, NIH
Yan Li	National Institutes of Health
Sara Lin	National Heart, Lung, and Blood Institute, NIH
Ti Lin	OD/NIH
Gabriel R. Linares	National Institutes of Health
Paul Liu	National Human Genome Research Institute, NIH

NIH Participants	
Chengyu Liu	National Institutes of Health
Justin Lowenthal	NIH Clinical Center, Department of Bioethics
Xinxing Lu	National Institute of Diabetes & Digestive & Kidney Diseases, NIH
Huiyan Lu	National Institute of Diabetes & Digestive & Kidney Diseases, NIH
NadyaLumelsky	National Institute of Dental and Craniofacial Research, NIH
Martha S. Lundberg	National Heart, Lung, and Blood Institute, NIH
Balazs Mayer	Adult Stem Cell Unit, NIDCR, NIH
Willie McCullough	Office of Research Infrastructure Programs, NIH
Sheldon Miller	National Eye Institute, NIH
Manuel Moro	National Institutes of Health
Stuart Moss	National Institute of Child Health and Human Development, NIH
Stefan Muljo	National Institute of Allergy and Infectious Diseases, NIH
Nancy Nadon	National Institute on Aging, NIH
Raymond O'Neill	OD, Division of Comparative Medicine, NIH
Svetlana G. Potapova	National Institute of Diabetes & Digestive & Kidney Diseases, NIH
Ravi Ravindranath	National Institute of Child Health and Human Development, NIH
Pamela Gehron Robey	CSDB, National Institute of Dental and Craniofacial Research, NIH
Kelly Robier	National Institute on Deafness and Other Communication Disorders, NIH
Soumen Roy	National Institute on Deafness and Other Communication Disorders, NIH
Milan Rusnak	National Institutes of Health
Paul Sammak	National Institutes of Health
Sheryl M. Sato	National Institutes of Health

NIH Participants	
Grace Shen	National Eye Institute, NIH
Felipe Sierra	National Institute on Aging, NIH
Hideko Takahasi	National Eye Institute, NIH
John Thomas	National Heart, Lung, and Blood Institute, NIH
SundarVenkatachalam	National Institutes of Health
Fei Wang	National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH
Hua Wang	National Institutes of Health
Lan-Hsiang Wang	National Heart, Lung and Blood Institute, NIH
Tongguay Wang	National Institute of Neurological Disorders and Stroke, NIH
Xiantao Wang	National Institutes of Health
Thomas Winkler	National Heart, Lung and Blood Institute, NIH
Baldwin Wong	National Institute on Deafness and Other Communication Disorders, NIH
Rosemary Wong	National Institutes of Health
Chuanfeng Wu	National Heart, Lung and Blood Institute, NIH
Koji Yoshinaga	National Institutes of Health
Troy Zarcone	National Institutes of Health
Connie Zhang	National Eye Institute, NIH
Zhen Zhang	National Institutes of Health
Zhensheng Zhang	National Institutes of Health

Non-NIH Participants	Institution
Kamal Ameis	Georgetown University
Patrick Au	Food and Drug Administration
Roger Avery	College of Veterinary Medicine, Virginia Tech
Jen Barrett	Virginia Tech
Steve Bauer	Food and Drug Administration
Justin Benjamin	Virginia Maryland Regional College of Veterinary Medicine
Karen Berry	California Institute For Regenerative Medicine (CIRM)
Lauren Black	Navigators, Charles River Laboratories
SmitaBhonsale	Armed Forces Institute of Regenerative Medicine
Richard Banas	Stemnion, Inc.
Mark Burke	Howard University School of Medicine
Cynthia Chang	Food and Drug Administration
Theresa Chen	Center for Biologics Evaluation and Research, FDA
Dena E. Cohen	Harvard University/HHMI
Eliza Curnow	University of Washington National Primate Research Center
Linda Dahlgren	Virginia-Maryland Regional College of Veterinary Medicine
Will Eyestone	Virginia-Maryland Regional College of Veterinary Medicine
Jia-Qiang He	Virginia Polytechnic Institute and State University
Mo Heidaran	Food and Drug Administration
Bill Huckle	College of Veterinary Medicine
Deb Hursh	Center for Biologics Evaluation and Research, FDA
Ping Jiang	The Wistar Institute
Helen Karuso	Regeneus Ltd.
SehwonKoh	North Carolina State University
Daekee Lee	EwhaWomans University
Ji-Hey Lim	College of Veterinary Medicine, NCSU

Non-NIH Participants	Institution
Jessica L. Lo Surdo	Center for Biologics Evaluation and Research, FDA
John McCarrey	University of Texas at San Antonio
Brent McCright	Center for Biologics Evaluation and Research, FDA
Malcolm Meyn	Stemnion, Inc.
Michael V. Mendicino	Food and Drug Administration
Chris Navara	University of Texas at San Antonio
Eric Ostertag	Transposagen Biopharmaceuticals Inc.
Wu Ou	Food and Drug Administration
Jorge Piedrahita	Center for Comparative Medicine and Translational Research, NCSU
SivashankarRamakrishnan	Virginia Maryland Regional College of Veterinary Medicine
Larisa Rudenko	Center for Veterinary Medicine, FDA
Michelle Theus	Virginia Tech
Mani Vessal	California Institute for Regenerative Medicine
Richard Vulliet	University of California at Davis
William Watsom	BTW Consulting
Allen Wensky	Food and Drug Administration
Diana M. Yoon	Food and Drug Administration

Appendix C. List of Poster Exhibits

May 23-24, 2012
Poster Exhibits

This list shows the six posters that were exhibited during the 2-day event. The number indicates the number on the poster board.

19) Ostertag, Eric M., New Advances for Transgenesis and Site-Specific Mutagenesis in the Rat.

21) Vulliet, P.R., et al. Alfie, the Wonder Cat, or Correcting In-born Errors of Metabolism with Adult Bone Marrow Stem Cells (fMSCs).

25) Navara, Christopher and McCarrey, John, PriStem, A primate Resource for Developing Stem Cell Therapies.

26) Koh, Sehwon, et al. Growth requirements and chromosomal instability of canine induced pluripotent stem cells.

27) Lim, Ji-hey, Naturally occurring spiral cord injury in dogs: a clinically relevant model of chronic paralysis in humans.

28) Lim, Ji-hey, Development of a model of sarcocaudal spinal cord injury in cloned Yucatan minipigs for cellular transplantation research.